American Journal of Medical Genetics 92:142–146 (2000)

X-Linkage Does Not Account for the Absence of Father–Son Similarity in Plasma Uric Acid Concentrations

Danielle R. Reed and R. Arlen Price*

Center for Neurobiology and Behavior, Department of Psychiatry, University of Pennsylvania, Philadelphia, Pennsylvania

Plasma uric acid concentration aggregates in families, and this similarity has been suggested to be due, in part, to multiple shared genes. Men have higher plasma uric acid concentrations than women and are affected with gout nine times more frequently. Rare forms of hyperuricemia and gout are due to mutations of X-linked genes (HPRT1 and PRPS1). Given these observations, we tested the hypothesis that normal variation in plasma uric acid levels would display a pattern of familial similarity consistent with X-linkage in 892 individuals from 196 obese but otherwise healthy families. As predicted by X-linked inheritance, fathers and sons showed no resemblance in plasma uric acid concentration (r = 0.013, NS), while all other pairings showed moderate-to-strong familial resemblance (ranging from 0.167, $P < 0.01$, parent–offspring to 0.415, sister–sister, $P < 0.01$). We then tested the hypothesis that loci along the X chromosome would influence plasma uric acid concentration. We conducted both single-point and multipoint linkage analyses using 17 X-linked markers spaced at approximately 9 cm intervals to determine whether allele sharing among sibs was related to sib similarity in plasma uric acid concentrations (n = 1,100 sib pairs). We found no regions of the X chromosome that cosegregated with plasma uric acid concentrations ($P > 0.05$). We conclude that variation in genes on the X chromosome contribute little to normal variation in plasma uric acid concentrations. Am. J. Med. Genet. 92:142–146, 2000.

KEY WORDS: uric acid; linkage; family study; X chromosome; complex traits

INTRODUCTION

Elevated plasma uric acid concentration (hyperuricemia) precedes the onset of gout. Uric acid is a metabolite of purine catabolism, and renal excretion is the primary route of elimination from the body. Elevated uric acid levels are increased after the ingestion of meat and other foods containing purines and after ingestion of alcohol. In addition to environmental determinants, twin and family studies have demonstrated the importance of shared genes in uric acid concentration [Boyle et al., 1967; Brunner et al., 1971; Dal Colletto et al., 1983; Feinleib et al., 1977; Friedlander et al., 1988; Gulbrandsen et al., 1979; Havlik et al., 1977; Jensen et al., 1965; Kalousdian et al., 1987; Laskarzewski et al., 1983; Morton 1979; Rao et al., 1982; Rice et al., 1990; Stecher et al., 1949; Whitfield and Martin 1983]. An early study suggested that gout is a single-gene disorder with an autosomal dominant mode of inheritance with incomplete penetrance [Stecher et al., 1949]. A reanalysis of these data and subsequent segregation analysis of additional families has determined that gout and hyperuriciemia are polygenic rather than monogenic traits [Friedlander et al., 1988; Gulbrandsen et al., 1979; Morton 1979; Rao et al., 1982]. This conclusion is further supported by the variability in metabolic disturbances of uric acid metabolism; no common biochemical abnormality is common to all hyperuricemic or gouty patients [Seegmiller et al., 1963].

Several lines of evidence suggest that some genes involved in uric acid regulation might reside on the X chromosome. First, gout and hyperuricemia are more common in men than women. The average level of uric acid is 1 mg/dl more in men compared to women and almost 95% of patients with gout are male [Bondy and Rosenberg, 1980]. Second, mothers transmit gout to

Grant sponsor: NIH; Grant numbers: R01DK44073, R01DK48095; Grant sponsor: US Public Health Service; Grant number: 1P41RR03655 from the National Center for Research Resources.

*Correspondence to: R. Arlen Price, Ph.D., Center for Neurobiology and Behavior, University of Pennsylvania, 415 Curie Blvd, CRB 135b, Philadelphia, PA 19104.
E-mail: arlen@bgl.psycha.upenn.edu

Received 10 November 1999; Accepted 25 January 2000

sons but fathers rarely do [Stecher et al., 1949]. Third, the patterns of correlations found among opposite-sex twin pairs are consistent with sex-linked inheritance [Whitfield and Martin, 1983]. Finally, rare forms of gout and hyperuricemia are associated with single mutations of X-linked genes (HPRT1 and PRPS1 [Becker et al., 1973; Kelley et al., 1967; Yen et al., 1978]). Common forms of gout and hyperuricemia appear to be determined by multiple genes, and one or more of these genes may reside on the X chromosome.

We tested this hypothesis by examining the pattern of family similarity in plasma uric acid concentrations and conducting quantitative linkage analysis in 892 family members from 196 families using 17 markers from the X chromosome. Some family members studied were obese but otherwise healthy, and families were unselected for a family history of gout or hyperuricemia.

MATERIALS AND METHODS

Families

We studied nuclear families ascertained from an ongoing family study at the University of Pennsylvania. Families were selected for study if at least two sibs were obese (one sib body mass index (BMI) $\geq$ 40 and one sib BMI $\geq$ 30) and the family contained at least one normal weight sib and one normal weight parent (BMI $\leq$ 27) [Price et al., 1998]. The resulting families had a wide range of obese and nonobese phenotypes. We divided four three-generation families into eight nuclear families.

Anthropometric and Plasma Uric Acid Measures

Subjects were weighed and measured for height without shoes, either in hospital gowns or light street clothing. Weight was measured with an Algen Corp Tri Coastal HS 600 scale to the nearest pound and height was measured with a portable Seritex stadiometer to the nearest 0.25 inch. Blood was drawn by venipuncture after an 8-hr fast and analyzed for plasma uric acid (Corning Clinical Laboratories, Horsham, PA). Each subject answered questions contained on a medical history survey.

Data Analysis, Descriptive Statistics, Univariate, and Familial Correlations

Descriptive statistics were computed for mothers, fathers, sisters, and brothers. Before analysis of family correlations or linkage analysis, plasma uric values were examined to determine if they covaried with age, sex, or BMI; if so, they were adjusted for these variables using multiple regression. Of the several indices available to adjust for obesity, we selected BMI because it accounted for the most variance in uric acid levels and was available for most subjects. Standardized residuals were used as the phenotype in all subsequent analysis of family similarity and linkage.

The correlation between subject characteristics and plasma uric acid concentrations were computed and examined (SPSS 6.1). Family correlations were computed using the FCOR program contained within the SAGE package (Case Western Reserve University, Cleveland). The reported correlations were computed giving equal weight to pairs.

DNA Analysis and Genotyping

DNA preparation. DNA was isolated from whole blood or immortalized lymphoblastoid cells using the high salt method [Lahiri and Nurnberger, 1991].

Genotyping. We selected 17 markers that spanned the X chromosome with an average interval of 9.3 cm and no gap greater than 13.5 cm (see Fig. 1 for the list of markers). PCR was conducted in a 10 μl reaction volume using conditions appropriate for the particular marker, using previously described protocols [Lee et al., 1999]. Alleles were scored by two readers, blind to phenotype, and checked for Mendelian incompatibilities using the computer program GENEHUNTER. Any incompatibilities identified in the original films were evaluated for scoring or data entry errors. Markers were rerun as required.

Data analysis, gene mapping. To estimate locus order and intermarker distance we used the map distances from the Whitehead Institute (WI) [Dib et al., 1996]. For markers not found in the WI database, we consulted the Genome Database, the Cooperative Human Linkage [Sheffield et al., 1995] database, and the Genetic Location Database [Collins et al., 1996]. (See the Electronic Database Information section for the URLs of these databases.)

We computed two statistics as implemented by MAPMAKER/SIBS version 2.0 [Kruglyak et al., 1996]: the EM algorithm-based Haseman-Elston regression (EMHE) [Haseman and Elston, 1972] and the nonparametric linkage quantitative trait loci analysis (NPL) [Kruglyak and Lander, 1995]. In contrast to the traditional Haseman-Elston regression, the EMHE uses information from the full IBD distribution when computing the regression between IBD and the squared phenotypic difference. NPL employs the Wilcoxon

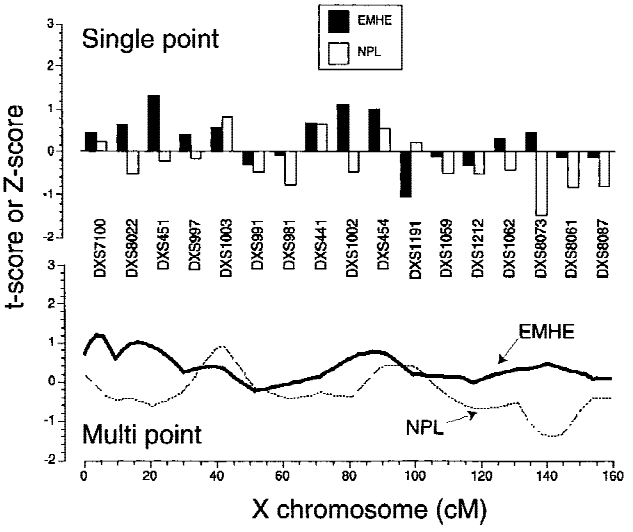

Fig. 1. Single point (upper) and multi point (lower) analysis of plasma uric acid concentration for markers along the X chromosome.

TABLE I. Age, BMI kg/m^2, Uric Acid Concentration of Family Members

Relative	n	Age (years)	BMI kg/m^2	Uric acid (mg/dl)
Mother	189	65 + 9 (41–84)	33.3 + 9.3 (18.8–65.8)	5.1 + 1.6 (1.6–12.9)
Father	113	68 ± 8 (42–86)	29.3 ± 6.0 (17.3–48.9)	5.7 ± 1.7 (2.6–14.0)
Index cases	204	39 ± 8 (16–61)	47.6 ± 8.9 (28.8–82.9)	5.2 ± 1.4 (2.0–9.3)
Sisters	347	39 ± 8 (17–59)	34.4 ± 9.9 (18.8–77.6)	4.4 ± 1.5 (1.4–11.2)
Brothers	194	39 ± 9 (15–65)	30.9 ± 7.6 (19.7–64.8)	6.0 ± 1.3 (2.8–8.9)

Uric acid values were not available for all family members ($N_{mothers}$ = 161, $N_{fathers}$ = 96, $N_{index\ cases}$ = 173, $N_{sisters}$ = 302, $N_{brothers}$ = 160).

rank-sum test to evaluate the relation between the rank of absolute phenotypic difference and IBD at a given locus. This approach is robust to assumptions of normality of squared phenotypic differences and lack of correlation between the differences and their residuals. We used all sib pairs in the analysis, and multiple sib pairs within families were weighted by $\Sigma(N\text{-}1)\text{-}2$ as suggested by Wilson and Elston [1993]. MAPMAKER/SIBS version 2.0 evaluates both autosomal and X-linked chromosome data; for X-linkage, it is assumed that the difference in phenotype is a function of whether zero or one alleles are inherited identical-by-descent from the mother [Cordell et al., 1995]. Note that parents and sibs providing DNA but without uric acid values were used in the analysis to provide additional information for the computation of identity-by-descent sharing.

RESULTS

Subject Characteristics

The parents were in their mid–late 60s and their children were in their late 30s to early 40s (Table I). The average BMI of all groups was over 30 kg/m^2 and ranged from 17.3–82.9 kg/m^2. Uric acid measurements were obtained on 892 of 1,047 family members (85%). Uric acid levels were, on average, highest in brothers and fathers and lowest in sisters and mothers and ranged from 1.4–14.0 mg/dl. Two individuals reported a previous diagnosis of gout, a father (uric acid 9.8 mg/dl) and a brother from a different family (uric acid 6.3 mg/dl).

Uric acid levels were dependent on sex, age, and BMI kg/m^2 (Table II). The relationships between sex, BMI, and uric acid were consistent for mothers, fathers, index cases, sisters, and brothers, but the effects of age were not the same for each group. There was a negative correlation between age and uric acid levels for some but not all groups; therefore, phenotypes were adjusted using multivariate regression within sex, generation, and index case categories.

TABLE II. Correlation Between Sex, Age, and BMI kg/m^2 With Uric Acid Concentration

Relationship	Sex	Age	BMI kg/m^2
All subjects	0.323**	–0.081*	0.290**
Mother	N/A	0.034	0.382**
Fathers	N/A	0.105	0.432**
Index case	0.230**	–0.104	0.336**
Sisters	N/A	0.085	0.518**
Brothers	N/A	–0.066	0.373**

N/A = not applicable.
*$P \leq 0.05$.
**$P \leq 0.01$.

Familial Aggregation

Plasma uric acid concentrations aggregated in families. There were significant correlations between parent–offspring pairs and among sibs (Table III), with the exception of fathers and their sons. The lack of father–son similarity was not due to the presence of extreme values. When all analyses were conducted eliminating uric acid values greater than 2 SD from the mean, the results were similar to those obtained using all phenotypes (data not shown). Spouse correlations did not differ from zero.

Gene Mapping Results

Multipoint and single point results for 1,100 sib pairs were in good agreement and both methods of analysis (EMHE and NPL) yielded similar results (Fig. 1). The highest t- or Z-score barely exceeded 1.0 for only one marker for the single-point analyses and a different marker for the multipoint analyses.

DISCUSSION

Plasma uric acid levels aggregated within families, with the exception of father–son pairs, which showed no similarity in plasma uric acid levels. While this observation might be explained by the absence of sharing between father and sons for genes on the X chromosome, the linkage results did not support this hypothesis. No linkage was observed between the X chromosome marker and uric acid concentrations ($P > 0.05$).

One previous attempt has been made to find linkage between polymorphic loci, gout, and hyperuricemia. In

TABLE III. Inter- and Intraclass Correlations Among Family Members for Uric Acid Concentration

Relationship	n	Correlation coefficients (r)
Mother–father	77	0.041
Parent–offspring	842	0.167**
Sib	863	0.349**
Mother–daughter	382	0.169**
Mother–son	142	0.189**
Father–daughter	232	0.201**
Father–son	86	0.013
Sister–sister	439	0.415**
Sister–brother	339	0.247**
Brother–brother	85	0.295**

Correlation coefficients marked with an * indicated statistically significant evidence for familial aggregation (* = $P < 0.05$; **$P < 0.01$).

black South Africans, one allele of the HLA system (HLA-B14) was more common in patients with primary gout compared with a control group, but the implications of this observation are unclear [Cassim et al., 1994].

An important question for multigenic traits is the extent to which genes that determine Mendelian disorders also have alleles that contribute to normal quantitative variation. Although two single gene mutations cause gout, neither of these genes appears to influence uric acid concentrations in our families. The placement of our markers relative to these genes makes it unlikely that the lack of support for linkage was due to low marker density or uninformative haplotypes (DXS1062—6 Mb—HPRT1—3.5 Mb—DXS8073; DXS1059—1.3 Mb—PRPS1—6.2 Mb—DXS1212).

Two other studies examined the pattern of family correlations for uric acid concentrations, finding that sons resembled their fathers nearly to the same extent that they resembled their mothers [Brunner et al., 1971; Friedlander et al., 1988]. In neither of these studies was uric acid concentration adjusted for BMI, so the father–son similarity could be secondary to resemblance in weight and height. Additionally, in both reports the sons were children, whereas in the current study the offspring were middle-aged. Because uric acid levels are influenced by sex hormones [Bondy and Rosenberg, 1980], different genes might be involved in uric acid concentration before and after puberty. Additional studies in families with adult children are needed to confirm the lack of father–son similarity found among the families studied here. Most family members have been genotyped for multiple autosomal markers (data not shown). Therefore, a bias in nonpaternity detection is unlikely to account for the lack of father–son similarity.

Environmental influences are present for most complex disorders such as diabetes and hypertension. In the case of gout and hyperuricemia, food selection and alcohol consumption have large effects on final uric acid concentration. While no adoption studies have been conducted, several lines of evidence suggest that a similarity in uric acid concentration between family members is not solely accounted for by similarity in diet. In one study of Israeli children, those living in collective housing (Kibbutz) were similar to their parents in uric acid concentration, although all families studied ate food prepared in a single kitchen [Brunner et al., 1971]. In another study, uric acid concentrations were more similar if adult twins lived together but similarity for twins living apart was still strong [Boyle et al., 1967]. Adoption studies and detailed studies of twins may help resolve the relative contribution of diet and genotype.

Besides sex, an important determinant of uric acid concentration is weight adjusted for height. As BMI increases, so does uric acid concentration, and this relationship is true for both men and women. In the analysis of family resemblance and linkage, the phenotypes were adjusted for BMI, and therefore genes with a pleiotropic effect on BMI and uric acid concentration would have been less likely to be found. However, analysis of uric acid concentrations unadjusted for BMI displayed no evidence for linkage (data not shown). The mechanism by which plasma uric acid concentration is increased among individuals with elevated BMI is not known, but leptin appears to have a role in the development of hyperuricemia [Fruehwald-Schultes et al., 1999].

ACKNOWLEDGMENTS

We thank the participating families for their generous cooperation. We also thank Robin Kilker, Sami Weisberg, Duc Bach, Richard Joseph, Nicholas Guido, Karynn Henry, Elizabeth Joe, Andrew C. Krakowski, Paula Kwon, Christa Alberry Mayr, Balasahib G. Shinde, and Guang Ming Yuan for technical assistance. Joseph H. Lee provided assistance in statistical analysis. This research was supported in part by NIH grants R01DK44073, and R01DK48095 (RAP). Some of the results of this article were obtained by using the program package SAGE, which is supported by a U.S. Public Health Service Resource Grant (1P41RR03655) from the National Center for Research Resources.

ELECTRONIC DATABASE INFORMATION

URLs for the data in this article are as follows:

Whitehead Institute, http://www-genome.wi.mit.edu
Genome Database, http://www.gdb.org
Cooperative Human Linkage Database, http://www.chlc.org
Genetic Location Database, http://cedar.genetics.soton.ac.uk

REFERENCES

Becker MA, Meyer LJ, Seegmiller JE. 1973. Gout with purine overproduction due to increased phosphoribosylpyrophosphate synthetase activity. Am J Med 55:232–242.

Bondy PK, Rosenberg LE. 1980. Metabolic control and disease. Philadelphia: WB Saunders.

Boyle JA, Greig WR, Jasani MK, Duncan A, Diver M, Buchanan WW. 1967. Relative roles of genetic and environmental factors in the control of serum uric acid levels in normouricaemic subjects. Ann Rheum Dis 26:234–238.

Brunner D, Altman S, Posner L, Bearman JE, Loebl K, Lewin S. 1971. Heredity, environment, serum lipoproteins and serum uric acid. A study in a community without familial eating pattern. J Chronic Dis 23:763–773.

Cassim B, Mody GM, Deenadayalu VK, Hammond MG. 1994. Gout in black South Africans: a clinical and genetic study. Ann Rheum Dis 53:759–762.

Collins A, Frezal J, Teague J, Morton N. 1996. A metric map of humans: 23,500 loci in 850 bands. Proc Natl Acad Sci USA 93:14771–14775.

Cordell HJ, Kawaguchi Y, Todd JA, Farrall M. 1995. An extension of the maximum Lod Score method to X-linked loci. Ann Hum Genet 59:435–449.

Dal Colletto GM, Krieger H, Magalhaes JR. 1983. Genetic and environmental determinants of 17 serum biochemical traits in Brazilian twins. Acta Genet Med Gemellol 32:23–29.

Dib C, Faure S, Fizames C, Samson D, Drouot N, Vignal A, Millasseau P, Marc S, Hazan J, Seboun E, Lathrop M, Gyapay G, Morissette J, Weissenbach J. 1996. A comprehensive genetic map of the human genome based on 5,264 microsatellites. Nature 380:152–154.

Feinleib M, Garrison RJ, Fabsitz R, Christian JC, Hrubec Z, Borhani NO, Kannel WB, Rosenman R, Schwartz JT, Wagner JO. 1977. The NHLBI twin study of cardiovascular disease risk factors: methodology and summary of results. Am J Epidemiol 106:284–285.

Friedlander Y, Kark JD, Stein Y. 1988. Family resemblance for serum uric acid in a Jerusalem sample of families. Hum Genet 79:58–63.

Fruehwald-Schultes B, Peters A, Kern W, Beyer J, Pfutzner A. 1999. Serum leptin is associated with serum uric acid concentrations in humans. Metabolism 48:677–680.

Gulbrandsen CL, Morton NE, Rao DC, Rhoads GG, Kagan A. 1979. Determinants of plasma uric acid. Hum Genet 50:307–312.

Haseman J, Elston R. 1972. The investigation of linkage between a quantitative trait and a marker locus. Behav Genet 2:3–19.

Havlik R, Garrison R, Fabsitz R, Feinleib M. 1977. Genetic variability of clinical chemical values. Clin Chem 23:659–662.

Jensen J, Blankenhorn DH, Chin HP, Sturgeon P, Ware AG. 1965. Serum lipids and serum uric acid in human twins. J Lipid Res 6:193–205.

Kalousdian S, Fabsitz R, Havlik R, Christian J, Rosenman R. 1987. Heritability of clinical chemistries in an older twin cohort: the NHLBI Twin Study. Genet Epidemiol 4:1–11.

Kelley WN, Rosenbloom FM, Henderson JF, Seegmiller JE. 1967. A specific enzyme defect in gout associated with overproduction of uric acid. Proc Natl Acad Sci USA 57:1735–1739.

Kruglyak L, Lander E. 1995. Complete multipoint sib-pair analysis of qualitative and quantitative traits. Am J Hum Genet 54:439–454.

Kruglyak L, Daly M, Reeve-Daly M, Lander E. 1996. Parametric and nonparametric linkage analysis: a unified multipoint approach. Am J Hum Genet 58:1347–1363.

Lahiri DK, Nurnberger JI. 1991. A rapid non-enzymatic method for the precipitation of HMW DNA from blood for RFLP studies. Nucleic Acids Res 19:5444.

Laskarzewski PM, Khoury P, Morrison JA, Kelly K, Glueck CJ. 1983. Familial hyper- and hypouricemias in random and hyperlipidemic recall cohorts: the Princeton School District Family Study. Metabolism 32:230–243.

Lee JH, Reed DR, Li W-D, Xu W, Joo E-J, Kilker RL, Nanthakumar E, North M, Sakul H, Bell C, Price RA. 1999. Genome scan for human obesity and linkage to markers in 20q13. Am J Hum Genet 64:196–209.

Morton NE. 1979. Genetics of hyperuricemia in families with gout. Am J Med Genet 4:103–106.

Price RA, Reed DR, Lee JH. 1998. Obesity related phenotypes in families selected for extreme obesity and leanness. Int J Obes 22:406–413.

Rao DC, Laskarzewski PM, Morrison JA, Khoury P, Kelly K, Glueck CJ. 1982. The Clinical Lipid Research Clinic Family Study: familial determinants of plasma uric acid. Hum Genet 60:257–261.

Rice T, Vogler GP, Perry TS, Laskarzewski PM, Province MA, Rao DC. 1990. Heterogeneity in the familial aggregation of fasting serum uric acid level in five North American populations: the Lipid Research Clinics Family Study. Am J Med Genet 36:219–225.

Seegmiller JE, Laster L, Howell RR. 1963. Biochemistry of uric acid and its relation to gout. N Engl J Med 268:712–827.

Sheffield V, Weber J, Buetow K, Murray J, Even D, Wiles K, Gastier J, Pulido JC, Yandava C, Sunden SL. 1995. A collection of tri- and tetranucleotide repeat markers used to generate high quality, high resolution human genome-wide linkage maps. Hum Mol Genet 4:1837–1844.

Stecher RM, Hersh AH, Solomon WM. 1949. The heredity of gout and its relationship to familial hyperuricemia. Ann Int Med 31:595–614.

Whitfield JB, Martin NG. 1983. Inheritance and alcohol as factors influencing plasma uric acid levels. Acta Genet Med Gemellol 32:117–126.

Wilson A, Elston R. 1993. Statistical validity of the Haseman-Elston sib-pair test in small samples. Genet Epidemiol 10:593–598.

Yen RC, Adams WB, Lazar C, Becker MA. 1978. Evidence for X-linkage of human phosphoribosylpyrophosphate synthetase. Proc Natl Acad Sci USA 75:482–485.